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Rapid Analysis of Illegal Cationic Dyes in Foods and Surface Waters Using High Temperature Direct Analysis in Real Time High-Resolution Mass Spectrometry

Ruizhi Wen[†,‡], Dong Zeng[§], Zihui Yang[†], Le Jiang[†], Ming Ma[†], Bo Chen[*,†] and Teris A. van Beek[*,ǀ]

[†] Key Laboratory of Phytochemical R&D of Hunan Province and Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University, Changsha 410081, China.

[‡] School of Sciences, Central South University of Forestry & Technology, Changsha 410004, China.

[§] Hunan Provincial Center for Disease Control and Prevention, Changsha 410005, China.

[ǀ] Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands.

[*]Corresponding Authors. E-mail address: dr-chenpo@vip.sina.com (B. Chen). Tel & Fax: +86-731-88872531.

ABSTRACT: A high temperature desorption (HTD) direct analysis in real time - high-resolution mass spectrometric (DART-HRMS) method was developed for the rapid analysis of four banned cationic dyes. Rhodamine B is used to dye foods, while malachite green, crystal violet and methylene blue are added to fishponds as antimicrobials. A simple induced phase separation extraction was used to pretreat samples. The DART-HRMS method employed two temperature steps, i.e., 200 °C for drying, purification and enrichment of sample solution and 500 °C for thermal desorption and ionization of analytes. The calibration curves of dyes in the range of 50-2000 ng/mL were linear using deuterated malachite green as an internal standard. The LODs vary for all analytes between 0.1-30 ppb depending on matrix and experimental conditions. Through analyses of real samples, two chili powders and one chili oil were found to be contaminated by rhodamine B. The concentrations were comparable with those found by an HPLC-MS/MS method.

KEYWORDS: illegal cationic dyes, direct analysis in real time, high-resolution mass spectrometry, induced phase separation extraction, rhodamine B, malachite green, crystal violet, methylene blue, food safety.

INTRODUCTION

A major food safety problem is the illegal addition of inedible or even toxic chemicals. Therefore, since 2008 the Chinese government has issued a list of food additives that may endanger Chinese food safety. Nowadays, 151 compounds occur on the Chinese joint high risk list (the so-called food additives black list).[1] They include 47 inedible components, 22 abused additives, and 82 prohibited drugs or components used in feed or drinking water for animals, or in livestock, poultry, and aquatic culture

processes. Every year, Chinese regulatory control departments examine all kinds of food samples on a large scale in order to make a preliminary assessment of the possible food safety risks.

Low-throughput analytical methods, like HPLC-MS and GC-MS, limit the number of samples analyzed and illegal additives frequently escape detection. Therefore, one of the major trends in food analysis is to develop relatively cheap, fast, sensitive, yet selective screening methods for the trace analysis of targeted and untargeted contaminants in foods. Only the positive samples then need to be re-analyzed by slower official methods. Flow injection analysis (FIA) MS is such a fast method but it suffers from a lack of enrichment options, ion suppression and possible contamination of the mass spectrometer. Ambient ionization MS (AIMS) is another example of a fast method and was first proposed in 2004.[2] Since then, more than 30 different ambient ionization techniques have been developed.[3] They can directly analyze different forms of samples including solids, semi-solids, and liquids under ambient air conditions with little or no sample pre-treatment. Among them, direct analysis in real time (DART)-MS is one of the more established techniques. It can be used for high-throughput food analysis,[4] including quality control and safety control[5] but also food authentication.[6,7]

DART is an atmospheric pressure chemical ionization (APCI)-related technique. The two major processes in DART are thermodesorption of analytes in a stream of a hot gas and APCI-like ionization.[8-10] Therefore, the temperature of the ionization gas is a key factor to obtain the required intensity of analyte ions. Often a balance must be considered between possible thermal degradation or pyrolysis of compounds, which results in a signal drop or even its disappearance, and on the other hand effective thermodesorption into the gaseous phase. Increasing the gas temperature up to 550 °C might be an option for detecting thermally stable but only marginally volatile components.

Marginally volatile chemicals, which can be encountered in foods, include synthetic cationic dyes. For instance, rhodamine B, **1**, is frequently used as an illegal food dye in seasonings and colored foods. In one survey, rhodamine B was detected in 26% of all investigated food samples.[11] In an Indian study even 50% of investigated chili and curry samples were found to be contaminated by **1** at ppm levels.[12] Rhodamine B has also been detected in foods imported in the UK.[13] Concentrations as high as 89 ppm have been reported.[14] Rhodamine B is considered to be potentially both genotoxic and carcinogenic.[15] Malachite green, **2**, crystal violet, **4**, and methylene blue, **5**, are used as antimicrobials in fishponds even though they have never been authorized for the use in fish for human consumption in the EU or USA. However, as they are potent, cheap and easy to use, they frequently end up in fish or fish products.[14,16] From 2002-2014, 548 non-compliant samples were encountered in the EU.[16] The reference point of action (RPA) for malachite green, **2**, is 2 µg/kg (2 ppb) in the EU,[16] however concentrations as high as 5000 ppb have been reported.[14] The toxicological effects of **2** have been reviewed[17,18] and it may be considered as carcinogenic and genotoxic *in vivo*.[16] The four dyes all occur on the Chinese food additives black list.[1] They are used for inappropriate economic interests although they are suspected to be carcinogenic, mutagenic, genotoxic, neurotoxic inducers in humans.[19-22] Recently a comprehensive review on all dyes including observed concentrations and analytical methods was published.[16]

DART-MS/MS has been used to analyze Sudan dyes in chili powders.[23] Cationic dyes are more difficult to analyze by an APCI-based ambient MS technique due to their poor volatility. For analyzing organic cationic chemicals, a reactive desorption corona beam ionization (DCBI)-MS, another type of APCI-based ambient MS technique, was developed. Quaternary amines could be detected at 220 °C. However, some byproducts might be produced.[24]

The aim of this study was to develop a highly sensitive DART-HRMS screening method working at high desorption temperatures for the analysis of illegal synthetic cationic dyes (Figure 1) in foods and fishpond water. The method was fully validated and applied on spiked and non-spiked commercial foods. The results show that this fast method can serve as an initial screening method instead of LC-MS.

MATERIALS AND METHODS

Chemicals and Materials. Rhodamine B chloride, **1**, malachite green oxalate, **2**, crystal violet chloride, **4**, methylene blue chloride, **5**, sodium chloride (Analytical grade), methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker Chemicals (Deventer, The Netherlands). Deuterated malachite green, **3**, was obtained from Witega (Berlin, Germany). Water (>18.2 MΩ) used for the experiments was purified by a Milli-Q System (Millipore, Bedford, MA).

Samples for spiking including wine, chili powder, chili sauce, chili oil, and soy sauce, were purchased from a local Dutch supermarket, and fishpond water was collected from local pools in Wageningen, the Netherlands. The pools contained various plants and were inhabited by fish and birds like ducks, coots and swans. Four Chinese chili powder samples and two chili oil samples including three samples contaminated by **1** were provided by Hunan Provincial Center for Disease Control and Prevention of China.

Standard and Sample Solutions Preparation for DART-HRMS Analysis. Individual stock solutions of **1**, **2**, **4** and **5** were prepared in methanol/water (1:1, v/v) at a concentration of 400 µg/mL. Stock solution of **3**, which was used as internal standard (I.S.) was prepared in methanol/water (1:1,

v/v) at a concentration of 10 μg/mL. Working standard solutions at 2000, 1000, 500, 200, 100, and 50 ng/mL were prepared in acetonitrile containing 100 ng/mL of I.S. The calibration curves were generated by plotting the ratio of the peak area of the analyte to that of the I.S. versus the concentrations of the analytes.

Sample solutions for analysis of **1** were prepared as follows: for wine and soy sauce samples, acetonitrile was added to the sample in a 1:1 volume ratio. After thorough mixing by shaking, the solution was placed in an ultrasonic bath for 5 min for extraction. An excess of solid sodium chloride was added to the mixture to induce phase separation. After centrifugation at 3000 rpm, the upper acetonitrile layer was collected and evaporated in an N_2 stream. The residue was redissolved in acetonitrile containing 100 ng/mL of I.S. The volume of acetonitrile was identical to the original volume of the sample.

For chili powder and chili sauce, 1.000 g of sample was extracted with 5.0 mL of water in an ultrasonic bath for 5 min. Then 5.0 mL of acetonitrile was added. After centrifugation at 3000 rpm, 3 mL of the liquid was taken, and its phase separation was induced by the addition of an excess of solid sodium chloride. After centrifugation at 3000 rpm, the upper acetonitrile layer was collected and evaporated in an N_2 stream. The residue was redissolved in 3.00 mL of acetonitrile containing 100 ng/mL of I.S.

For chili oil, 1.000 g of chili oil was dissolved in 4.0 mL of hexane. The hexane solution was extracted 3 times with acetonitrile/water (1:1, v/v), each time with 4 mL. The three aqueous acetonitrile extracts were combined, and phase separation was induced by adding an excess of solid sodium chloride. After centrifugation at 3000 rpm, the upper acetonitrile layer was collected and evaporated in an N_2 stream. The residue was redissolved in 4.00 mL of acetonitrile containing 100 ng/mL of I.S.

Sample solutions for the analysis of **2**, **4** and **5** were prepared as follows: 5 mL of acetonitrile was added to 5 mL of fishpond water. After thorough mixing by shaking, the sample was extracted in an ultrasonic bath for 5 min. An excess of solid sodium chloride was added to the solution to induce phase separation. After centrifugation at 3000 rpm, the upper layer was collected and evaporated in an N_2 stream. The residue was redissolved in acetonitrile containing 100 ng/mL of I.S. The volume was the same as that of the original water sample.

DART-Orbitrap MS Conditions. A DART-SVP (simplified voltage and pressure) ion source (IonSense, Saugus, MA) was coupled to an Exactive orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA). All full-scan measurements were performed with a scan range of *m/z* 100.0-1000.0 in positive mode, a mass resolution of 100,000 (full width at half-maximum, FWHM), and a maximum injection time of 100 msec. The DART-SVP source was operated in positive-ion mode and a temperature setting of 200 °C for drying and sample clean-up, and 500 °C for desorption and ionization unless indicated otherwise. All samples were analyzed in transmission mode with a Linear Rail Controls (IonSense) at a scan speed of 0.5 mm/s. As ionization gas, helium was used at a flow rate of ~3.7 L/min. For normal analysis, 10 μL of sample solution was applied on metal mesh. After drying at 200 °C, 10 μL of water-methanol (1:1) was added to the sample spot by a 10 μL HPLC syringe. Afterwards the mesh with sample was analyzed at 500 °C by DART-HRMS. For DART-MS/HRMS analysis, a Q-Exactive mass spectrometer (Thermo Fisher Scientific) was operated in full scan (HRFS) (70,000 FWHM) and product scan (HRPS) (17,500 FWHM) modes.

Large Volume Sample Loading for DART. For the screening of low concentration samples, a large volume (50 or 100 μL) loading was employed. The DART source was operated in standby state with N_2 at a flow rate of ~3.5 L/min at 200 °C. 10 μL of sample solution was added 5 or 10 times on

metal mesh and repeatedly dried at 200 °C. Finally, 10 μL of water/methanol (1:1, v/v) was added to the dry sample spot, which was then analyzed at 500 °C.

RESULTS AND DISCUSSION

Optimization of DART Conditions. As a first step towards the development of a DART screening method for cationic dyes, it was investigated if all four dyes could be analyzed at all by DART and to this end four sampling modes were evaluated. Results were compared with ESI-MS infusion measurements on the same mass spectrometer (data not shown). Next optimization took place, paying attention to such factors as nature of gas used in DART, temperature settings, sample drying and wetting, and distances from sample to MS inlet and DART exit. These experiments are discussed as follows:

Effect of sampling mode on DART-HRMS. For DART-MS analysis, samples can be introduced by means of different devices such as a glass dip-it, a hollow glass capillary for applying samples on thin layer chromatography (TLC) plates, a triangular filter paper or a metal mesh. The different sample introduction modes were compared by loading 10 μL of 100 ng/mL mixed standard solution on the different devices. Metal mesh gave the most stable MS signal. The signal was also strong due to the large surface area in transmission mode. When a dip-it or a TLC application capillary loading were moved in the DART sampling zone, the solvent present more or less exploded due to the high temperature, and the signal was highly unstable. When using triangular filter paper, the sensitivity was low because the gas must flow around the paper instead of through the paper.

Effects of gas temperatures and gas type on DART-MS responses. The temperature of the gas exiting the DART is one of the key factors in DART-MS analyses. Normally, the relationship between

168 signal intensity vs. gas temperature shows a bell-shaped curve (maximum response between 200-
169 400 °C). The final results depend on the physico-chemical properties of the analytes. For thermally
170 stable marginally-volatile compounds, increasing the gas temperature setting above 400 °C up to the
171 maximum setting of 550 °C might be beneficial.[9,25] To investigate the relationship between responses
172 of the dyes and gas temperature, standard solutions of 100 ng/mL were tested on the metal mesh at
173 different temperatures. The results are shown in Figure 2. Different from normal DART temperature
174 curves, optimal temperatures for the dyes are 450-550 °C. Below 400 °C, there was almost no response.
175 The optimal temperature for methylene blue, **5**, is slightly lower than that for the other three dyes. This
176 reflects the higher volatility of **5**, which is related to its lower molecular weight. At 500 °C, the
177 responses of the dyes reached a plateau. Therefore, to get good responses of the dyes, the gas
178 temperature was set to 500 °C in all further experiments.

179 In addition, the gas influences the DART-MS ionization. When N_2 was compared with He as
180 working gas at 200, 300, 400 and 500 °C, none of the four dyes showed any MS response with N_2,
181 ruling out its use as ionization gas. However, as there is no evaporation below 300 °C (Figure 2) and
182 evaporation is not gas-dependent, both N_2 and He can be safely used as a drying gas at 200 °C to
183 remove solvent and more volatile interferents in sample solutions and after large volume sampling, i.e.,
184 it does not cause any loss of analytes. N_2 was chosen for drying as it is the cheaper of the two gases.
185 Figure 3 shows the results of large volume loading to increase sensitivity. Between 29-34 min, 5 µL of
186 10 ng/mL was loaded 10 times, each time followed by drying at 200 °C. Between 39.4-40.2 min, 10
187 µL of water/methanol (1:1, v/v) was added to the combined dried sample spot, which was then
188 analyzed at 500 °C.

Effect of adding solvent to the sample on DART-HRMS responses at 500 °C. When analyzing at 500 °C, there was no response if no solvent was added to the sample spot after drying. The major hurdle for DART-HRMS analysis is low volatility. Actually, the gas temperature at the sampling spot, because of mixing with ambient air, is much lower than that of the set value of the DART temperature controller. When the temperature is set at 450 °C, the actual temperature at the sample spot is only ~200 °C.[26] Therefore, the thermal desorption of these dyes when dry is difficult. When solvent was added, the rapid evaporation of the solvent, which is present in large excess, presumably assists in the transfer of dye molecules to the gas phase. Therefore, the addition of a solvent to the sample spot prior to the DART measurement at 500 °C is a prerequisite for these analytes. The less-volatile analytes desorb from a tiny droplet through the assistance of explosive solvent evaporation at the last moment of liquid evaporation.[27-30]

Effect of sample position on DART-HRMS responses. There are two distances, which affect the sensitivity and must be optimized in DART-MS analyses. One is the distance between the exit nozzle of the DART and sample, and the second is the distance between sample and cone of the mass spectrometer. In this experiment, samples were loaded on the metal mesh of the motorized DART rail. Therefore, the positions of the DART source and the motorized rail determine the distances.

For the distance between sample and cone of the mass spectrometer, there are just two fixed distances, i.e., large (about 20 mm) and short (about 5 mm) because of two fixed positions of the motorized rail. The results of measuring a 100 ng/mL mixed standard solution at both distances are shown in Figure 4. During 60.25-62 min, the samples were at a large distance from the MS cone. During 65.8-67.6 min and 70-71.8 min, the samples were at a short distance. The sensitivity at the

large distance was much higher than that at the short distance. Therefore, all following experiments were performed at the large distance.

For the distance between the exit nozzle of the DART and the sample on the mesh, a higher sensitivity was obtained at a shorter distance because the temperature of DART gas is then highest due to less mixing with the ambient atmosphere. The following experiments were all carried out at a distance of 5 mm.

Sample Pretreatment. For the analysis of **1**, **2**, **4** and **5** in foods and water, a number of methods have been published. Most often an HPLC-based method was chosen,[31-34] and sample pretreatment is crucial. Solid-phase extraction (SPE), membrane filtration, and gel permeation chromatography (GPC) have been employed frequently.[35] However, all are time-consuming and labor-intensive. Induced phase separation extraction (IPSE) is a relatively new technique belonging to the class of liquid-liquid extraction (LLE) techniques. IPSE consists of extraction of target compounds by a mixed solvent, then adding a phase-separation inducer to induce phase separation and quantitative migration of the analytes to one of the phases. IPSE has an advantage that the resulting separation of the solvents is easier than with classical LLE, especially in the presence of emulsion-forming impurities. In addition, the extraction efficiency of the process may be higher than that of a traditional liquid-liquid extraction.[36] Overall, it is an alternative simple sample pretreatment method, which has been used for different types of samples.[37,38] Thus IPSE is well compatible with DART analyses and its usefulness for purifying cationic dyes was investigated. In combination with acetonitrile and water as extraction solvents, three inducers (sodium chloride, potassium sulfate and potassium chloride) were compared. When using sodium chloride as inducer, the phase separation was completed in one minute. The extraction recovery was 97%, 94%, 94%, and 93% for **1**, **2**, **4**, and **5**, respectively. The four dyes all stayed in the acetonitrile

phase. With the other two inducers, phase separation was incomplete and the extraction efficiency was lower than 30%. For wine, chili sauce, and soy sauce samples, after IPSE, the contained proteins, salts and sugars were all present in the aqueous phase. In case of chili powder samples, the acetonitrile phase was primrose yellow and the aqueous phase deep red. A large amount of water-soluble natural pigment in chili remained in the aqueous phase and was thus removed from the dyes.

Effect of preheating samples at 200 °C. The effect of heating the sample at 200 °C with nitrogen versus a direct analysis at 500 °C with helium was studied by comparing the mass spectra of spiked chili sauce (Figure 5). Clearly the pretreatment at 200 °C removed low molecular weight background interferences from the sample. The beneficial effect of such a "thermal separation" to facilitate DART of mixtures has been reported previously.[39] While the 200 °C pretreatment slightly increased the total time of the DART analysis, still the analysis of 10 samples could be completed within 10 min with the automated Linear Rail add-on (140 mm in length) at a scan speed of 0.5 mm/sec.

Mass resolution. As food matrixes are very complex, there are large numbers of compounds in the sample solutions, including interfering isobaric contaminants.[40] As in ambient MS there is no chromatographic separation, a very high mass resolution is required for obtaining sufficient selectivity. For the analysis of rhodamine B, **1**, based on the mass spectra, soy sauce was the most complex sample matrix. Figure 6 shows three isobaric contaminants in the mass spectrum of soy sauce between *m/z* 443.1 and *m/z* 443.4. To resolve the one at *m/z* 443.2115 from **1** at *m/z* 443.2325, a mass resolution of at least 22,000 is required. The used orbitrap mass spectrometer has a mass resolution of ~90,000 in this mass spectrum, i.e., it provides sufficient selectivity. The various fishpond samples were much cleaner, i.e., no isobaric matrix components were visible in blanks within 0.2 Da of the molecular ions of malachite green, **2**, crystal violet, **4**, and methylene blue, **5**.

Quantitative Analysis by DART-HRMS. Due to the intrinsic short-term fluctuations of any DART-MS signal, good quantitative results can only be obtained by an internal standard having similar physical and chemical properties. Therefore, an isotope internal standard, deuterated malachite green (**3**), was chosen. The calibration curves were generated by plotting the ratio of the peak area of the analyte to that of the IS versus the concentrations. Working standard solutions at 50, 100, 200, 500, 1000, and 2000 ng/mL were prepared in acetonitrile with 100 ng/mL of IS. Four calibration curves with correlation coefficients (R^2) higher than 0.99 were obtained, showing that **3** also worked well as an internal standard for **1**, **4** and **5**. The reproducibility was measured at all concentrations of the calibration curve and all RSDs (n=3) were lower than 13%.

Three chili powder samples were purchased from a local Dutch supermarket, and three fishpond water samples were collected from local pools in Wageningen. Rhodamine B, **1**, could not be detected in any of the foods nor any of the dyes **2**, **4** and **5** in fishpond waters. Next, the recovery of the proposed IPSE-HTD-DART-HRMS approach was determined by spiking experiments. Method reproducibility and recovery are listed in Table 1. Good recoveries for all four dyes at three concentrations were obtained, ranging from 87.2-118.5% while the repeatability was good (RSD <21%, n=3).

Under optimized conditions, the LODs were investigated by adding **1** to different food matrixes and **2**, **4** and **5** to fishpond water. The LOD for **2**, **4** and **5** in fishpond water is 10 ppb when using the Exactive MS and applying 10 µL IPSE sample on the mesh. For **1**, the LOD is dependent on the dilution and matrix. It is 10 µg/kg for wine and soy sauce, 25 µg/kg for chili powder and chili sauce, and 30 µg/kg for chili oil, respectively. When applying 100 µL on the mesh, these values are tenfold lower, 1~3 ppb. Thus, not for all matrixes the RPA of 2 ppb can be reached with this set-up, however with a more sophisticated mass spectrometer (*vide infra*), the RPA can be met with certainty.

In the literature, comparisons of the performance of different mass analyzers have been published.[41,42] High-resolution MS detection often exhibited better selectivity than triple-quadrupole MS due to the removal of isobaric species. This was the case with[41] and without chromatography.[42] MS/HRMS methods showed even better selectivity as well as increased sensitivity.[41,42] Although the selectivity of our DART-HRMS method was sufficient, preliminarily experiments with a Q Exactive Focus Hybrid Quadrupole-Orbitrap mass spectrometer coupled to DART were carried out to observe the effect on method sensitivity. It was approximately tenfold higher and concentrations as low as 0.1 ng/mL (0.1 ppb), could be detected by making use of the MS/HRMS option, i.e., by measuring a specific daughter ion after fragmentation. In combination with the inherent higher selectivity, it implies that a DART quadrupole orbitrap MS set-up could be used to analyze more complex samples, e.g., fish tissues, with lower concentrations of the cationic dyes.

Application. Next the final method was applied on real samples. The samples included two wines, three chili oils, five chili powders, two chili sauces, two soy sauces, and three fishpond waters. Among those samples, four chili powders and two chili oils were provided by China (HPCDCPC). Two Chinese chili powders and one Chinese chili oil were contaminated by rhodamine B, **1**. The concentrations according to IPSE-HTD-DART-HRMS were 0.870 ± 0.007 μg/g and 2.13 ± 0.02 μg/g in the two chili powders, and 4.53 ± 0.02 μg/g in the chili oil. According to standard HPLC-MS/MS analyses carried out by HPCDCPC, the concentrations were 1.08 μg/g and 1.78 μg/g in the two chili powders and 4.37 μg/g in the chili oil respectively. The other three Chinese samples were negative according to both HPLC-MS/MS and IPSE-HTD-DART-HRMS analyses. Thus, the results show an excellent correspondence between the official HPLC-based methodology and the fast DART-MS

297 method presented here. The minor differences could be caused by the heterogeneity of samples, storage
298 or the very different methodology.

299 Overall, the newly developed validated IPSE-HTD-DART-HRMS method could serve as a fast
300 screening method for cationic dyes. Advantages over a more traditional HPLC-UV approach of food
301 dyes (LOD~1 ppm),[43] include rapidity, lack of a chromatographic step and 1000-fold higher sensitivity.
302 Disadvantages are higher equipment and maintenance costs and higher required expertise. Rhodamine
303 B, **1**, can be detected in a variety of aqueous and oily foods and the sensitivity of 1 ppb seems more
304 than sufficient as in the three commercial foods in which **1** was detected, the concentrations were in
305 the range of 1-2 ppm, i.e., three orders of magnitude larger than the detection limit when using 100 µL
306 sample loading in combination with the standard Orbitrap MS. Although for these samples there was
307 excellent correspondence with HPLC analyses, positive samples should be confirmed by HPLC
308 analyses. However, samples, which do not contain **1** according to IPSE-HTD-DART-HRMS screening,
309 do not need to be analyzed by HPLC. Assuming that currently the majority of commercial samples is
310 not contaminated, this means many more samples could be checked by food authorities when using
311 the proposed fast screening methodology. Although the method might be applied on fish samples, this
312 was not tested. We propose to use the method for screening commercial fishponds for the presence of
313 **2**, **4** and **5**. Due to the great sensitivity, with a Q-Exactive as MS and 100 µL loading as low as 0.1 ppb,
314 it should be easy to detect which fishponds are free of cationic dyes. Fishes from fish ponds
315 contaminated with **2**, **4** or **5** are suspect and should be checked. Again, this should lead to a higher food
316 safety. As the raw data can be interrogated retrospectively, the methodology could also be used for
317 checking the presence of other banned substances with similar properties in a non-targeted fashion.[44]

In other words, the scope of the proposed methodology might be actually wider than the four cationic dyes.

ABBREVIATIONS

AIMS, ambient mass spectrometry; APCI, atmospheric pressure chemical ionization; DART-HRMS, direct analysis in real time - high-resolution mass spectrometry; DCBI, desorption corona beam ionization; FIA, flow injection analysis; FWHM, full width at half-maximum; GPC, gel permeation chromatography; HPCDCPC, Hunan Provincial Center for Disease Control and Prevention of China; HRFS, high resolution full scan; HRPS, high resolution product scan; HTD, high temperature desorption; IPSE, induced phase separation extraction; LLE, liquid-liquid extraction; RPA, reference point of action; SPE, solid phase extraction; SVP, simplified voltage and pressure.

SUPPORTING INFORMATION*

Figure SI-1. Diagram of distances of nozzle of DART with sample and sample with cone of MS

Figure SI-2. Photos of motorized rail at different positions

Figure SI-3. Calibration curves including equation and correlation coefficients (n=3)

Figure SI-4. DART-Q Exactive MS HRPS detection of 1 ng/mL of **1**

Figure SI-5. DART-Q Exactive MS HRPS detection of 1 ng/mL of **2**

Figure SI-6. DART-Q Exactive MS HRPS detection of 1 ng/mL of **3**

Figure SI-7. DART-Q Exactive MS HRPS detection of 1 ng/mL of **4**

Figure SI-8. DART-Q Exactive MS HRPS detection of 1 ng/mL of **5**

Figure SI-9. DART-Q Exactive MS HRPS detection of 0.1 ng/mL of **1**, **2**, **4** and **5** standard solutions

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AUTHOR INFORMATION

Alternate corresponding author:

*: Teris A. van Beek. Address: Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands. E-mail: teris.vanbeek@wur.nl (T. A. van Beek). Tel: +31-317-482376. Fax: +31-317-484914.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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FIGURE CAPTIONS

Figure 1. Chemical structures of synthetic cationic dyes. Accurate masses: **1**, ([M]$^+$**:** *m/z* 443.2335; **2,** [M]$^+$: *m/z* 329.2018; **3**, [M]$^+$: *m/z* 334.2332; **4**, [M]$^+$: *m/z* 372.2440; **5**, [M]$^+$: *m/z* 284.1221.

Figure 2. Ion abundances of 100 ng/mL solutions of **1**, **2**, **4** and **5** at different DART gas temperature settings. Note the mass range: **1**, [M]$^+$**:** *m/z* 443.23-443.24**; 2,** [M]$^+$: *m/z* 329.20-443.21; **4**, [M]$^+$: *m/z* 372.24-372.25; **5**, [M]$^+$: *m/z* 284.12-284.13.

Figure 3. DART ion chronograms of large volume loading (10× 10 μL of 10 ng/mL dye solution) at 200 °C (29-34 min) and subsequent analysis at 500 °C (39.4-40.2 min).

Figure 4. Ion abundances of a 100 ng/mL mixed standard solution of **1**, **2**, **4** and **5** at different distances between sample and cone of MS. Note mass range: **1**, [M]$^+$**:** *m/z* 443.23-443.24**; 2,** [M]$^+$: *m/z* 329.20-443.21; **3**, [M]$^+$: *m/z* 334.23-334.24; **4**, [M]$^+$: *m/z* 372.24-372.25; **5**, [M]$^+$: *m/z* 284.12-284.13.

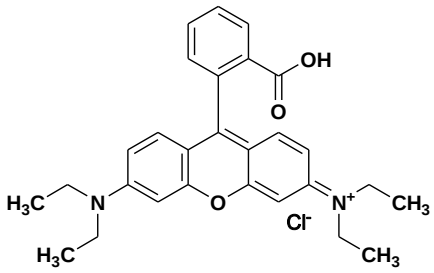
Figure 5. Comparison DART full scan mass spectra of chili sauce containing 100 ng/g of **1**: (A) direct analysis at 500 °C; (B) 200 °C pretreatment before analysis at 500 °C.

Figure 6. DART – orbitrap mass spectra from *m/z* 443.1-443.4 of soy sauce sample (A) blank, and (B) spiked at 100 ng/g with rhodamine B, **1**.

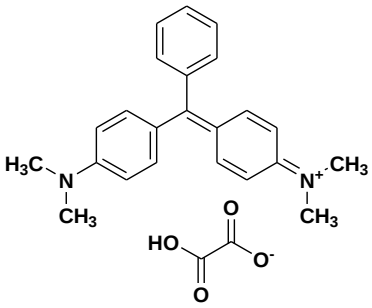
Table 1 Accuracy and precision of the IPSE-HTD-DART-HRMS method

Dye	Spiked samples (ng/g)	Calculated concentration (ng/g)	RSD (n=3)	Recovery (%)
1[b]	0	ND[a]	ND	ND
	200	237	0.19	118.5
	500	458	0.11	91.9
	1000	872	0.18	87.2
2[c]	0	ND	ND	ND
	200	206	0.05	103.0
	500	506	0.07	101.2
	1000	967	0.07	96.7
4[c]	0	ND	ND	ND
	200	210	0.07	105.0
	500	486	0.04	97.2
	1000	1045	0.09	104.5
5[c]	0	ND	ND	ND
	200	217	0.11	108.5
	500	535	0.09	107.0
	1000	917	0.14	91.7

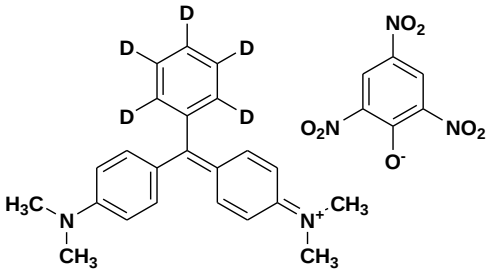
[a] ND: not detected. [b] chili powder. [c] fishpond water



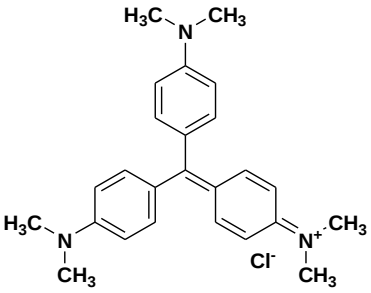
Rhodamine B (**1**)



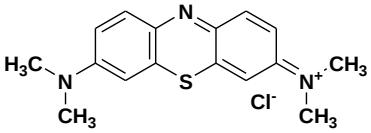
Malachite green (**2**)



Deuterated malachite green (**3**)



Crystal violet (**4**)



Methylene blue (**5**)

Figure 1.

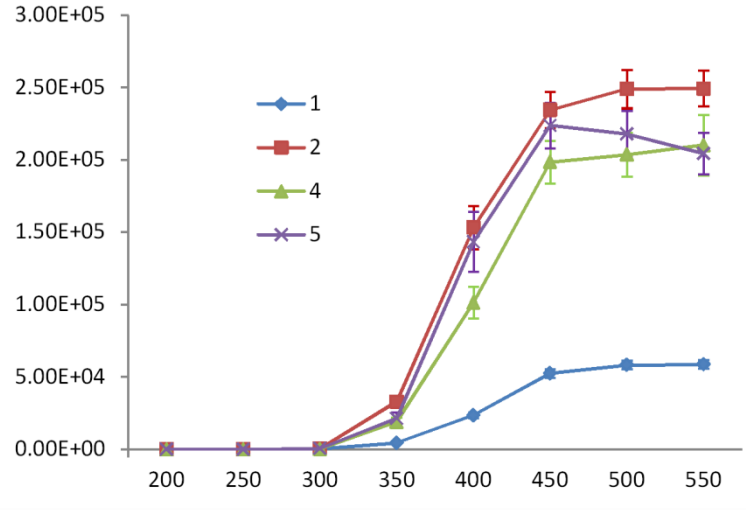
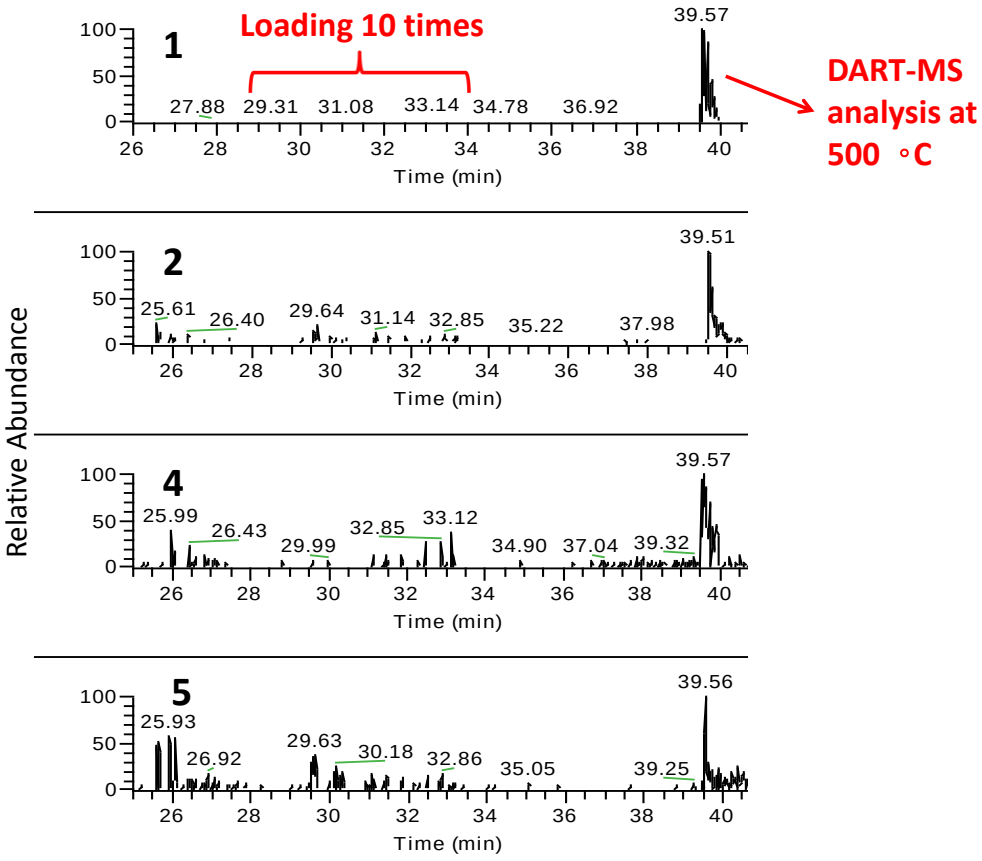

3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
200
250
300
350
400
450
500
550
1
2
4
5

Figure 2.


Loading 10 times
DART-MS analysis at 500 ◦C
Relative Abundance
Time (min)
1
2
4
5
27.88
29.31
31.08
33.14
34.78
36.92
39.57
25.61
26.40
29.64
31.14
32.85
35.22
37.98
39.51
25.99
26.43
29.99
32.85
33.12
34.90
37.04
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30.18
32.86
35.05
39.25
39.56

Figure 3.

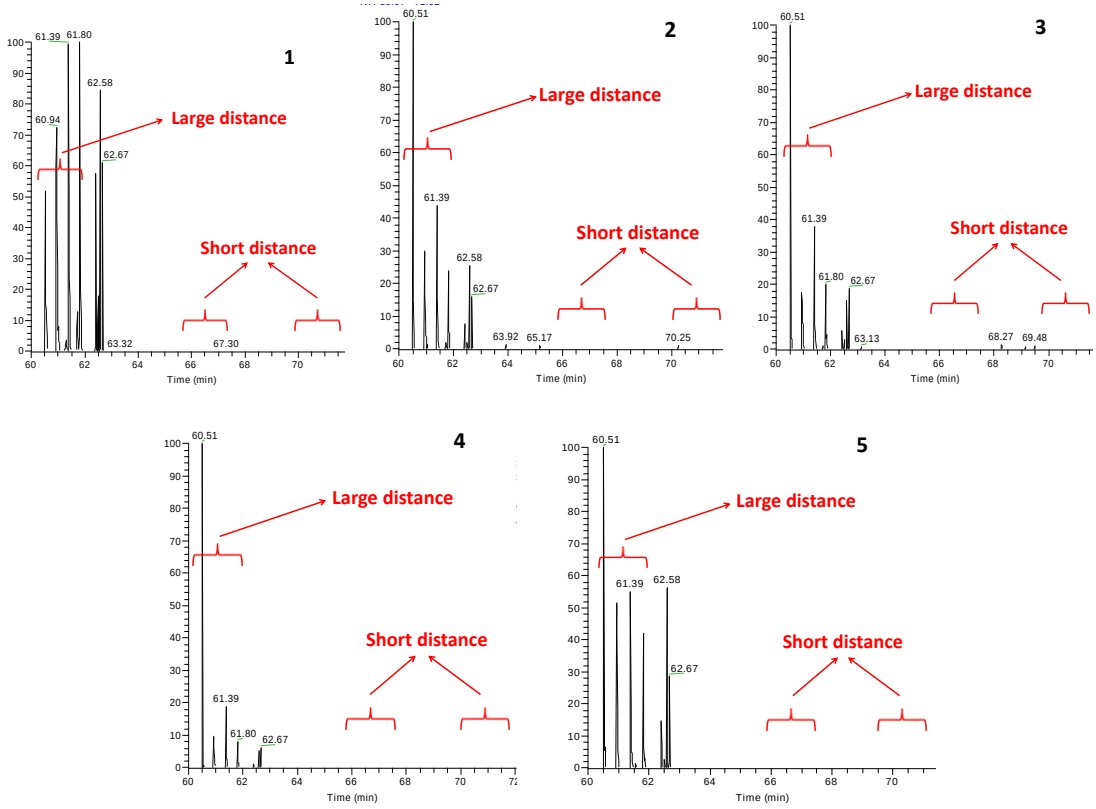
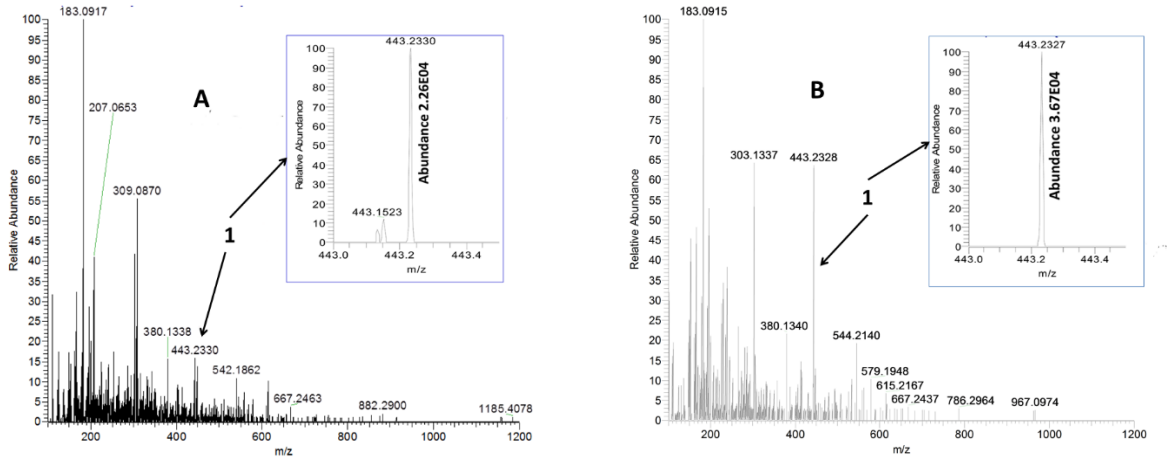

1
Large distance
Short distance
61.39
61.80
62.58
60.94
62.67
63.32
67.30
2
Large distance
Short distance
60.51
61.39
62.58
62.67
63.92
65.17
70.25
3
Large distance
Short distance
60.51
61.39
61.80
62.67
63.13
68.27
69.48
4
Large distance
Short distance
60.51
61.39
61.80
62.67
5
Large distance
Short distance
60.51
61.39
62.58
62.67
Time (min)

Figure 4.


A
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207.0653
309.0870
380.1338
443.2330
542.1862
667.2463
882.2900
1185.4078
1
443.2330
443.1523
Abundance 2.26E04
Relative Abundance
m/z
B
183.0915
303.1337
443.2328
380.1340
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443.2327
Abundance 3.67E04

Figure 5.

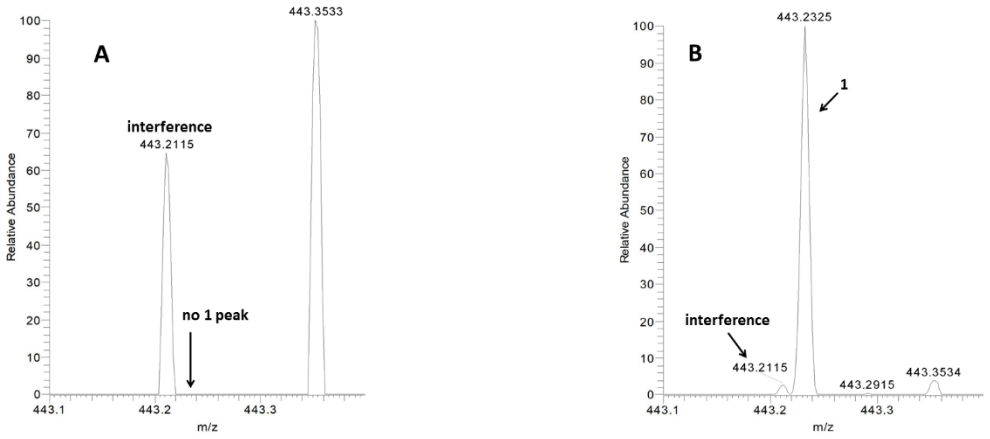
A
443.3533
interference
443.2115
no 1 peak
Relative Abundance
m/z
B
443.2325
1
interference
443.2115
443.2915
443.3534
Relative Abundance
m/z

Figure 6.

Graphical Abstract for Table of Contents